Abstract

Protein-protein interactions play a critical role in the functioning and regulation of several biological cellular processes. The analysis of these interactions facilitates the understanding of the mechanisms that trigger the progression of several diseases. Hence, the detection of protein and the study of affinity and binding kinetics of protein-protein interaction is of vital importance for the development of therapeutic drugs, medical diagnosis etc. The conventional methods for the detection of proteins such as Radioimmunoassay (RIA), Enzyme-inked Immunosorbent Assay (ELISA), Western Blot etc. despite being highly sensitive, generally require large volumes of reagents, sophisticated instruments, complex and skilled laboratory procedures characterized by a long time-to-result, labelling tags etc. On the contrary, Surface Plasmon Resonance (SPR) provides a non-invasive, label-free means to study the affinity and binding kinetics of biomolecular interactions involving proteins, lipids etc. The Refractive Index (RI)-sensing transduction mechanism of SPR provides rapid, sensitive and real-time information, without the need for washing and labelling steps, requiring minute amounts of analytes facilitating clinical applications.

The design and fabrication of SPR biosensors to investigate protein-protein interaction is presented in this thesis. SPR sensors based on multi-layer mathematical models were designed using an optimal choice of materials with appropriate design parameters such as RI, layer thickness etc. to achieve enhanced plasmonic performance. For the fabrication of SPR biosensors, a novel bonding protocol was used to bond PDMS based microfluidic channels with gold coated glass substrates. Polydimethylsiloxane (PDMS) based microfluidic channels were used to immobilize polyclonal antibodies raised in mouse, goat and rabbit for the construction of a multiple protein-patterned SPR biosensor and exposed to a target Human Immunoglobulin-G (H-IgG) protein. The measurements were performed using a custom-made SPR measurement setup in the Kretschmann configuration. The SPR biosensors were structurally characterized using Ultraviolet-Visible (UV-Vis) spectroscopy and the sensing performance was determined in terms of sensitivity, selectivity and Limit of Detection (LoD). Following the successful proof of concept with standard proteins, SPR biosensors were fabricated on gold coated glass substrates for the detection of crude snake venom protein. The biosensors exhibited high sensitivities (9.01678˚/(μg/ml) and 10.32268˚/(μg/ml)) and

low LoDs (9.37 ng/ml and 9.89 ng/ml) towards Indian cobra (*Naja naja*) and Indian Russell's viper (*Daboia russelii*) snake venoms respectively with a wide linear response (10 ng/ml-900 ng/ml, R^2=0.99) and a response time of ~16-20 min.

The conducted research further investigated atmospheric pressure based plasma treatment technique for the development of hydrophilic PDMS microchannels. The effect of the plasma treatment on the wettability, surface energy and hydrophilicity retention capacity of PDMS was determined using contact angle measurements and Fourier Transform Infrared (FTIR) analysis, whereas the bulk structural property and optical transparency were studied using X-Ray Diffraction (XRD) and UV-Vis Spectroscopy respectively. The plasma treated PDMS was used for the fabrication of an SPR biosensor and the results were compared with an SPR biosensor fabricated with pristine PDMS. The plasma treated SPR biosensor demonstrated very low LoDs of 7.13 ng/ml, 5.91 ng/ml and 1.47 ng/ml and high sensitivities of 16.8˚/(µg/ml), 13.5˚/(µg/ml) and 16.65˚/(µg/ml) against anti-Human Immunoglobulin-G protein raised in mouse, goat and rabbit respectively as compared to pristine SPR biosensor.

Keywords: *Surface plasmon resonance*; *refractive index*; *polydimethylsiloxane*; *microfluidic*; *Human Immunoglobulin-G*